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GUVs Melt Like LUVs: The Large Heat Capacity of MLVs Is Not Due to Large Size or Small Curvature

Mark A. Kreutzberger,[1] Emmanuel Tejada,[1] Ying Wang,[2] and Paulo F. Almeida[1,*]

[1]Department of Chemistry and Biochemistry, University of North Carolina Wilmington, Wilmington, North Carolina; and [2]Materials Processing Center, Massachusetts Institute of Technology, Cambridge, Massachusetts

ABSTRACT The excess heat capacity functions (ΔC_p) associated with the main phase transition of large unilamellar vesicles (LUVs) and multilamellar vesicles (MLVs) are very different. Two explanations are possible. First, the difference in vesicle size (curvature) results in different gel-fluid interactions in the membrane; those interactions have a large effect on the cooperativity of the phase transition. Second, there is communication between the bilayers in an MLV when they undergo the gel-fluid transition; this communication results in thermodynamic coupling of the phase transitions of the bilayers in the MLV and, consequently, in an apparent increase in the cooperativity of the transition. To test these hypotheses, differential scanning calorimetry was performed on giant unilamellar vesicles (GUVs) of pure dipalmitoylphosphatidylcholine. The ΔC_p curve of GUVs was found to resemble that of the much smaller LUVs. The transition in GUVs and LUVs is much broader (half-width ~1.5°C) than in MLVs (~0.1°C). This similarity in GUVs and LUVs indicates that their size has little effect on gel-fluid interactions in the phase transition. The result suggests that coupling between the transitions in the bilayers of an MLV is responsible for their apparent higher cooperativity in melting.

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*Correspondence: almeidap@uncw.edu

Phospholipid multilamellar vesicles (MLVs) undergo a much more cooperative gel-fluid phase transition than large unilamellar vesicles (LUVs) (1–3). The question is why. MLVs are very large but heterogeneous, with diameters $d \sim 1$–50 μm, and contain multiple bilayers in each vesicle (4). Large unilamellar vesicles (LUVs) are much smaller, with $d \sim 100$ nm, but fairly uniform, with a single bilayer (3). Two hypotheses can explain the disparity in the cooperativity of their phase transitions: 1) The difference in the size of the vesicles, and consequent difference in curvature, may result in a different interaction between gel and fluid lipids within each bilayer, which may be more unfavorable in MLVs than in LUVs; or 2) The high cooperativity of MLVs may be due to coupling between bilayers, in the third dimension, which would therefore undergo correlated melting.

To decide between these two alternatives, we measured the excess heat capacity ΔC_p associated with the main phase transition of giant unilamellar vesicles (GUVs) of DPPC (dipalmitoylphosphatidylcholine) by differential scanning calorimetry (DSC). In our preparations, typical DPPC GUVs have diameters $d \sim 5$–10 μm (Fig. 1), similar to MLVs and much larger than LUVs; but like LUVs, GUVs have a single bilayer. Our main result is shown in Fig. 2. The phase transition of MLVs is extremely cooperative (*green curve*), but GUVs (*black curve*) melt essentially like LUVs (*red curve*), and very differently from MLVs. Therefore, the highly cooperative melting of MLVs is not due to the large size or small curvature of each membrane in the vesicle. For easy reference, the thermodynamic parameters of the DPPC phase transition observed by DSC in vesicles of different types and sizes are listed in Table 1.

DPPC GUVs were prepared by electroformation in a 0.1 M sucrose solution, at 50°C, as previously described in Wheaten et al. (5) and Svetlovics et al. (6). The effect of 0.1 M sucrose on the phase transition of DPPC is negligible (7). The GUVs were visualized by confocal fluorescence microscopy with a Fluoview FV1000 microscope (Olympus, Melville, NY) in a solution of 0.1 M glucose and 50 μM carboxyfluorescein (Fig. 1). They appeared essentially identical upon heating to 60°C and cooling back to room temperature. The GUV size distribution determined by microscopy is shown in Fig. 3; the average diameter was 6.5 μm. The vesicle size distribution was further examined by dynamic light scattering (DLS). The size of the GUVs is too large to be accurately determined by DLS. However, it is clear that most of the scattering intensity comes from one main type of vesicles, which have small diffusion coefficients, corresponding to hydrodynamic radii >1 μm (Fig. S1 in the Supporting Material).

Several GUV preparations were necessary to obtain the amount of material needed for one DSC experiment (~50 μg of lipid). Four independent experiments were performed, in a high-sensitivity Nano Differential Scanning

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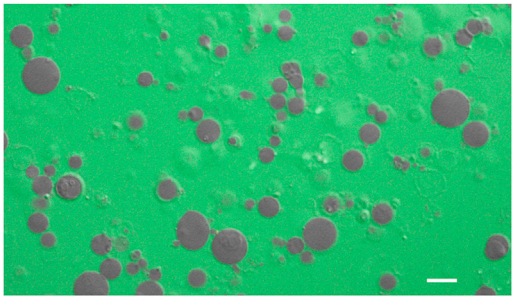

FIGURE 1 DPPC GUVs at room temperature. The membrane-impermeant green fluorescent dye carboxyfluorescein was added to the exterior solution for visualization. Scale bar, 10 μm. To see this figure in color, go online.

Calorimeter (TA instruments, New Castle, DE), as previously described in Svetlovics et al. (6). The scan rate was 0.1°C/min. We estimate the lipid concentration in the GUV samples to be ~50–100 μM from the number of GUVs in the microscope field, and mainly from the area under the ΔC_p peaks assuming that the enthalpy change (ΔH = 8.7 kcal/mol) is the same as in other types of DPPC vesicles (2,6,8–10). The results of the DSC experiments are shown in Fig. 4. The black curve is the average (also shown in Fig. 2) and the color curves are individual heating scans.

The excess heat capacity curve of DPPC GUVs (~6 μm) is similar to that of the much smaller LUVs (100 nm), both of which have only one bilayer. In contrast, the heat capacity peak of MLVs, which are heterogeneous and contain a variable number of bilayers, is much higher and narrower (Fig. 2 and Table 1). Further, there is no indication of a pretransition in the GUV scans (Fig. 4).

Lipid interactions within a bilayer can be quantified, in a two-state model, by the gel-fluid interaction parameter ω_{AB},

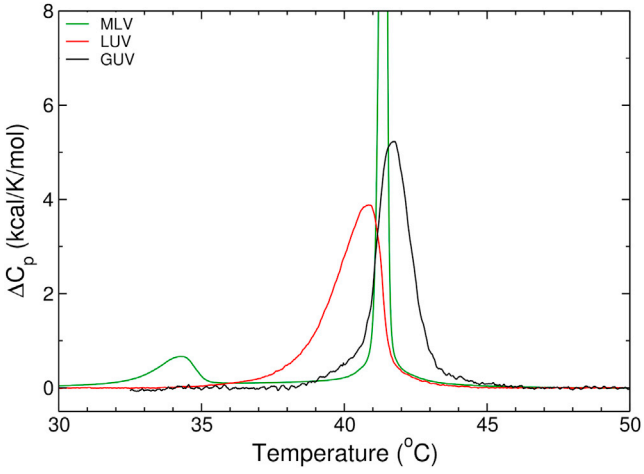

FIGURE 2 Excess heat capacity curves of DPPC GUVs (*black*), MLVs (*green*), and LUVs (*red*). The curves were slightly renormalized to a common ΔH = 8.7 kcal/mol and centered on the average T_m of each type of vesicle (listed in Table 1). To see this figure in color, go online.

TABLE 1 Size and thermodynamic parameters for different types of DPPC vesicles

Vesicle	Diameter	T_m [a]	δ_T [b]	ΔH [c]	ΔC_p [d]	ω_{AB} [e]
MLV	1–10 μm	41.4	0.1	8.7	100	380
LUV	100 nm	40.8	1.5	8.7	3.5	300
SUV	25 nm	37.2	3.0	8.7	2.0	280
GUV	5–10 μm	41.7	1.5	8.7	5.0	310[f]

[a]T_m in °C. The values listed for LUVs (~30 measurements), prepared by extrusion, and GUVs (seven measurements) are from our data. Published values for LUVs are T_m = 41.3 ± 0.1°C (2,14).

[b]Full width at half-height in °C. The value for MLVs at extremely low scan rates was δ_T = 0.076°C at 0.1°C/h (1) and δ_T = 0.096°C at 0.3°C/h (2). We routinely obtain δ_T = 0.2°C at 0.1°C/min.

[c]ΔH = 8.7 kcal/mol has been used as standard value. For LUVs we obtained ΔH = 8.7 ± 1.1 kcal/mol (~30 measurements). Published values of ΔH for MLVs and LUVs typically vary between ≈7.5 and 8.7 kcal/mol (2,14) and are typically lower for small unilamellar vesicles (SUVs), ≈6.0 kcal/mol. However, Monte Carlo simulations suggest that the true measure is ΔH = 8.7 kcal/mol, essentially independent of vesicle type (2,8,11). The lower values reported for SUVs are likely due to baseline uncertainty in the DSC curves.

[d]ΔC_p in kcal/K/mol. The values for MLVs vary widely in the literature, between ~10 and 100 kcal/K/mol (see Biltonen (1), Ivanova and Heimburg (2), Suurkuusk et al. (10), and references therein); the largest value (listed) is probably the most accurate. The value of 3.5 kcal/K/mol for LUVs is from our data (mean of ~30 measurements), consistent with 3.1 kcal/K/mol from Ivanova and Heimburg (2). Values for SUVs are ~1.5–2.0 (2,8). The value for GUVs is from our data, assuming ΔH = 8.7 kcal/mol.

[e]ω_{AB} in cal/mol, from Ivanova and Heimburg (2), Jerala et al. (8), and Almeida (11). Because ω_{AB} is very sensitive to the value of ΔC_p, the uncertainty is largest for MLVs (~±20 cal/mol). The values for LUVs and SUVs from different laboratories agree within ±4 cal/mol.

[f]Preliminary Monte Carlo simulations as described by Svetlovics et al. (6) (see the Note Added in Proof).

defined by $\omega_{AB} = \epsilon_{AB} - (1/2)(\epsilon_{AA} + \epsilon_{BB})$, where the ϵ values are the interaction free energies between lipid neighbors in the gel (A) and fluid (B) states. The parameter ω_{AB} has been determined by Monte Carlo simulations to match the heat capacity in the transition region (2,6,8,11). Positive values of ω_{AB}

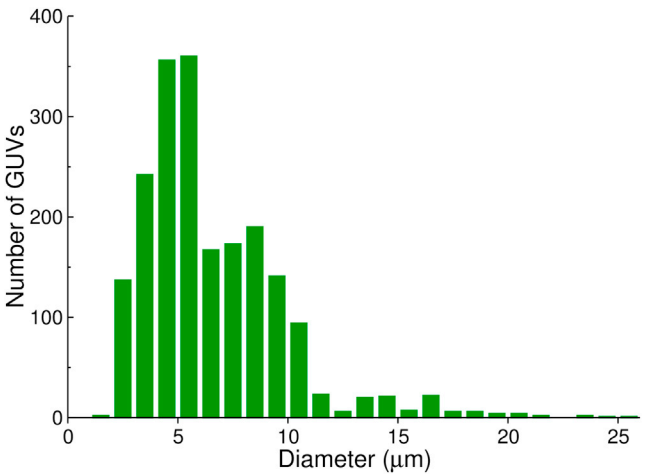

FIGURE 3 Size distribution of DPPC GUVs determined by microscopy in 2000 GUVs from three independent samples. To see this figure in color, go online.

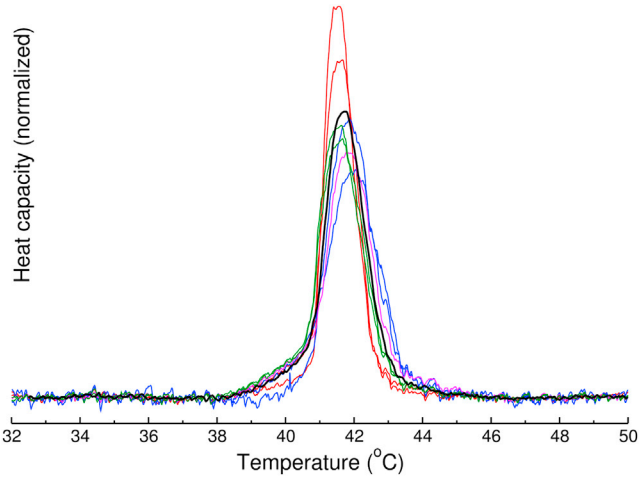

FIGURE 4 Heat capacity curves of DPPC GUVs obtained by DSC. The scans for four independent samples are shown in color. Consecutive heating scans (at a rate of 0.1°C/min, separated by cooling at 1°C/min) on the same sample are shown in the same color. All curves are normalized to the same area (enthalpy). (*Black curve*) Average of these seven scans. To calculate the average, the individual curves were first normalized to the same enthalpy and centered on the mean T_m = 41.7 ± 0.2°C. The average scan is the GUV curve shown in Fig. 2. To see this figure in color, go online.

indicate that the gel-fluid interaction is unfavorable (i.e., repulsive) compared to gel-gel and fluid-fluid interactions. In MLVs, where $\Delta C_p(T_m) \approx 100$ kcal/K/mol, $\omega_{AB} = 380$ cal/mol (1,2). In LUVs, where $\Delta C_p(T_m) \approx 3.5$ kcal/K/mol, $\omega_{AB} = 300$ cal/mol (2,6). This difference in ω_{AB} could reflect a real difference in interactions in LUVs and MLVs. However, based on our results, we conclude that the large ω_{AB} in MLVs does not reflect stronger repulsive gel-fluid interactions within a bilayer. Rather, we posit that the higher cooperativity is due to coupling of the phase transitions of the various bilayers in an MLV. This coupling probably does not occur by direct contact between adjacent bilayers. The lamellar spacing in fully hydrated multilayers of DPPC is ~65 Å (12). In the gel phase, this width is composed of a relatively thick membrane (52 Å) separated from the next by a thin water layer (11 Å); in the fluid phase, the lipid membrane becomes thinner (46 Å) and the water layer, thicker (20 Å). The area expansion that accompanies melting of the bilayers may be the cause of the phase transition coupling in MLVs. Alternatively, it is possible that the apparent higher cooperativity of the phase transition in MLVs may be due to inhibition of curvature fluctuations by adjacent bilayers in each vesicle (13).

Understanding the effect of vesicle size and interbilayer coupling on the phase transition of phospholipids is significant for investigations that use model membranes in protein-lipid interactions, membrane protein function, mechanisms of antimicrobial and cell-penetrating peptides (5), and the lipid lateral organization in membranes. GUVs are good models of plasma membranes, because of their large size and low curvature. However, most lipid-protein interactions have been studied in LUVs, which are slightly more strained, and, earlier, in MLVs. It is important to know which conclusions from experiments in single bilayers (LUVs) or in multibilayers (MLVs) are transferable to GUVs. The behavior of ΔC_p about the main phase transition is especially sensitive to interactions in the membrane. Its similarity in GUVs and LUVs supports the idea that experiments in single bilayers in LUVs and GUVs are comparable. The lipid molecules do not appear to behave differently in these two types of membranes.

SUPPORTING MATERIAL

Dynamic light scattering experiments, including one figure are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(15)00452-X.

AUTHOR CONTRIBUTIONS

M.A.K. performed and analyzed GUV microscopy and DSC; E.T. performed and analyzed LUV and MLV DSC; Y.W. performed and interpreted DLS; and P.F.A. designed research, analyzed data, and wrote the article.

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NOTE ADDED IN PROOF

Since the submission of this manuscript, preliminary Monte Carlo simulations performed in our laboratory, via standard methods (6), yielded a value of 310 cal/mol for the gel-fluid interaction in DPPC GUVs.